CCFA MODIFIED

INTENDED USE
Remel CCFA Modified is a solid medium recommended for use in qualitative procedures for selective and differential isolation of *Clostridium difficile*.

SUMMARY AND EXPLANATION
C. difficile was first isolated in 1935 and until the 1970s it was believed to be nonpathogenic for humans.[1] In the late 1970s, *C. difficile* was identified as the major cause of pseudomembranous colitis (antibiotic-associated colitis) or CDAD (*C. difficile*-associated disease).[2,3] In 1979, George et al. recommended the use of CCFA Agar, a solid medium containing cycloserine, cefoxitin, and fructose (CCFA), for isolation of *C. difficile* [4] CCFA Modified agar is based on the formulation of George et al. but it does not contain egg yolk for detection of lecithinase and lipase. *C. difficile* is lecithinase and lipase negative and CCFA Modified is inhibitory to other clostridia.

PRINCIPLE
Proteose peptone supplies carbon, nitrogen, vitamins, and minerals required for the growth of *C. difficile.* Amino acids are metabolized by *C. difficile* causing a rise in the pH, which changes the colonies and surrounding medium from red to yellow. Fructose is a carbohydrate source of energy. Sodium chloride and magnesium chloride are essential electrolytes which help maintain osmotic equilibrium. Disodium phosphate and monopotassium phosphate are buffers and sources of phosphate. Cycloserine and cefoxitin are selective agents. Cycloserine inhibits gram-negative bacilli and streptococci. Cefoxitin is a broad spectrum antibiotic which inhibits gram-positive and gram-negative bacteria, with the exception of *C. difficile* and *Enterococcus faecalis*. *C. difficile* will demonstrate yellow fluorescence on this medium when examined under longwave ultraviolet light.

REAGENTS (CLASSICAL FORMULA)*
Proteose Peptone.. 40.0 g
Fructose .... 6.0 g
Disodium Phosphate .... 5.0 g
Sodium Chloride .... 2.0 g
Monopotassium Phosphate .... 1.0 g
Magnesium Chloride .... 0.1 g
Neutral Red.. 0.03 g
Cycloserine .... 250.0 mg
Cefoxitin .... 16.0 mg
Agar .... 20.0 g
Demineralized Water .... 1000.0 ml

pH 7.6 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prior to use, reduce the plates for a minimum of 24 hours by placing them in an anaerobic environment at room temperature.
2. Inoculate specimens for anaerobic culture on both selective and nonselective media as soon as possible after receipt in the laboratory; streak plates for isolation.
3. Incubate anaerobically for 48-72 hours at 33-37°C.
4. Following incubation, observe the plate for growth of flat, circular colonies with filamentous edges, which demonstrate a yellow zone 2-3 mm out from the edge of the colony.
5. Inspect suspicious colonies under longwave ultraviolet light for yellow fluorescence.
6. Confirm anaerobic growth by Gram stain and subculture to a blood agar plate incubated in ambient air.
7. Consult appropriate references for additional tests to confirm the identification of *C. difficile*.[5-8]

QUALITY CONTROL
All lot numbers of CCFA Modified have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Clostridium difficile ATCC® 9689	Anaerobic, 48 h @ 33-37°C	Yellow colonies, yellow zone, yellow fluorescence
Bacteroides fragilis ATCC® 25285	Anaerobic, 48 h @ 33-37°C	Inhibition (partial to complete)
Clostridium perfringens ATCC® 13124	Anaerobic, 48 h @ 33-37°C	Inhibition (partial to complete)
Escherichia coli ATCC® 25922	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)
Staphylococcus aureus ATCC® 25923	Ambient, 18-24 h @ 33-37°C	Inhibition (partial to complete)

LIMITATIONS
1. Other organisms will grow on this medium but they do not form colonies with the characteristics described for *C. difficile*.
2. Typical Gram stain morphology of *C. difficile* may not be evident in colonies selected from this medium because of the antibiotics it contains. Subculture to nonselective anaerobic blood agar to obtain characteristic morphology.[7]

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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